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11223430 BIOSIS NO.: 199800004762

Construction and characterization of Escherichia coli O157:H7 strains expressing firefly luciferase and green fluorescent protein and their use in survival studies.

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1997

JOURNAL: Journal of Food Protection 60 (10):p1167-1173 Oct., 1997

ISSN: 0362-028X

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The firefly (*Photinus pyralis*) luciferase (luc) gene on plasmid vector pBESTluc and the *Aequorea victoria* green fluorescent protein (gfp) gene on plasmid vector pGFP were introduced into strains of *Escherichia coli* O157:H7. The recombinant *E. coli* strains were indistinguishable from their parent strains in biochemical and immunological assays and in a multiplex PCR reaction. There was no significant difference in the growth kinetics of the luc-bearing recombinants and the parent strains. At 37degreeC all of the recombinant strains maintained the vectors and expressed luciferase and the green fluorescent protein when grown both with and without antibiotic selection. Individual colonies of luc-bearing *E. coli* strains were readily luminescent in the dark after being sprayed

with a solution of 1 mM beetle luciferin. The recombinants containing pGFP emitted bright green fluorescence when excited with UV light and the addition of any other proteins, substrates, or cofactors was not required. The green fluorescent protein-expressing *E. coli* O157:H7 strains were used in studies examining the survival of the organism in apple cider and in orange juice. In apple cider the organism declined to undetectable levels in 24 days at refrigeration temperature while in orange juice the strains survived with only small decreases in number during the 24-day sampling period. These recombinant *E. coli* O157:H7 strains, containing readily identifiable and stable markers, could be useful as positive controls in microbial assays as well as in studies monitoring bacterial survival and the behavior of *E. coli* O157:H7 in foods and in a food processing environment.

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Fratamico, Pina M.; Deng, Ming Y.; Strobauch, Terence P.; Palumbo, Samuel A. (Microbial Food Safety Research Unit, Eastern Regional Research Center, Agricultural Research Service, U. S. Department Agriculture, Wyndmoor, PA 19038, USA). J. Food Prot., 60(10), 1167-1173 (English) 1997 International Association of Milk, Food and Environmental Sanitarians. CODEN: JFPRDR. ISSN: 0362-028X. DOCUMENT TYPE: Journal CA Section: 10 (Microbial, Algal, and Fungal Biochemistry) Section cross-reference(s): 17

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#### Keywords

luciferase recombinant *Escherichia* food marker  
fluorescent protein recombinant *Escherichia* food marker

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